1947

A Pathological Investigation of the Lake Poinsett Fish Disaster

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A PATHOLOGICAL INVESTIGATION OF THE LAKE POINSETT FISH DISASTER

By

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A thesis submitted to the Graduate Committee, South Dakota State College of Agriculture and Mechanic Arts, in partial fulfillment of the requirements for the degree of Master of Science.

May 1947

INTRODUCTION

During the first week of April, 1946, the ice cover of Lake Poinsett, South Dakota began breaking up and many thousands of dead fish appeared on the surface of the water. This was the beginning of a spectacle never before witnessed in this territory. Strong April winds swept ice fragments and dead fish in to the lake shores and within a few days there were from three to five tons of dead fish on every fifty feet of shore line and a like number of dead fish remained floating in the water nearby.

The immediate concern of the citizens residing in the vicinity of the lake was the disposition of the huge mass of dead fish. The situation rapidly became critical. There was considerable confusion and indecision. News of the situation was of top interest in the newspapers, and motorists came from miles around to witness the spectacle. The Lake Poinsett Region Association, of which Mr. Guy Abbott of Arlington, South Dakota was secretary, took initial control of the situation. The officers of the Brookings County Board of Health and the South Dakota State Board of Health were notified and familiarized with the details. Naturally, it was assumed by the Lake Region Association, that the South Dakota State Department of Game, Fish and Parks would be the organization responsible in such a matter. The department was notified; it denied responsibility and relegated

such duties to the State Board of Health. It was impossible for the State Board of Health to handle a situation of the kind as no funds were provided for emergency incidents of this nature. Pressure was brought to bear on the State Department of Game Fish and Parks and this organization finally took the responsibility of cleaning up the lake and disposing of the dead fish.

The State Highway Department brought road building equipment, bull-dozers and trucks to the lake. Many privately owned trucks in the immediate area were requisitioned and several hundred men were hired. Huge pits were excavated and the dead fish were hauled into these and covered with earth. Rendering plants in the locality removed as many as was possible for them to handle. Contact was made with Colonel Green, Army Engineer of Omaha, Nebraska, concerning the possibility of burning the fish, using the new jelly gasoline incendiary material. This incendiary compound had been used during the war with excellent results. The material was transported immediately to the lake along with a small contingent of men from an army depot in Colorado. The material was tested on a small stack of dead fish on the lake shore and the results were completely unsatisfactory. The work progressed, even though the obnoxious putrefactive odors made things unpleasant for the workmen. By May tenth the fish were entirely disposed of and the lake was again

restored to order.

The writer had the good fortune of being able to visit the lake during the week of April 8th to April 14th, immediately after the news of the situation was proclaimed. The fish were to be seen massed in piles along the lake shore and floating in the water, in some areas as far out in the lake as one could see. The fish were more numerous in some areas of the lake shore than in others. By reason of personal interests, the writer made preliminary investigations on the dead fish massed in several areas on the southern beach of the lake. Several hundred dead fish were counted and it was noted that approximately 98 percent were comprised of rough fish. The carp species were predominant. There were very few specimens of game fish noted. The writer examined many individual specimens of dead fish for appearance of pathological lesions. Several were dissected by means of a pocket knife. They were found to be in an advanced stage of putrefaction. There were no external lesions noted on any of the specimens examined. One interesting detail noted was the accumulation of a large number of air-sacs from fish on two particular areas of the lake; one, about five hundred square yards in scope, contained a large mass of these sacs floating on the surface of the water.

The writer was informed that samples of the dead carp

were sent to the state laboratory, University of South Dakota, and to the University of Minnesota. The results were negative. Saprification was so far advanced that detection of anything pathological was impossible.

It is quite possible that the fish had died a considerable period of time prior to the time the ice cleared off of the lake. This was indicated by the stage of putrefaction , a process which would naturally be inhibited by the low temperature of the water.

The writer made a hike around the south shore of Lake Poinsett on an afternoon of August, 1946. Several dead fish of the carp and bullhead species were noticed lying on the water's edge where they had been washed in from the lake. These specimens appeared to have been dead only a short period of time as saprification and the distinct putrefactive odors were absent. It seemed rather unusual to find samples of carp and bullhead as all of these were reported to have perished in April. Inquiry was made of several people living on the lake shore. The writer was informed that a few dead fish had been found on the lake shore during the summer months, but no game fish had been seen and there had been no game fish angled. The writer became interested in making a pathological investigation of the dead and diseased fish that were being washed in from the lake during the remainder of the summer and early

fall. The writer believed that if these fish were found to be killed by disease then perhaps the condition could be linked with the disaster of the preceding spring.

Plans were made during the month of September, 1946, to carry out a bacteriological investigation of the pathological aspects of the fish disaster of Lake Poinsett. Dead fish would be secured from the lake shore and examined for pathological lesions. Attempt would be made to isolate pathogenic bacteria from these lesions and grow them in pure culture. Identifications would be made and tests would be conducted to confirm their pathogenicity.

It was rumored at the time that the lake would be seined during the winter months in order to remove all of the remaining rough fish. Plans were made to be present at the lake during this operation in order to secure both live and dead samples of fish. However, these plans were cancelled a few months later when the seining program was vetoed by the Lake Poinsett Region Association.

A BRIEF SURVEY OF THE FIELD OF FISH PATHOLOGY

Very few exhaustive investigations have been conducted on the diseases of inland fish. It is practically a virgin field. Nearly all records found in literature dealing with the subject concern investigations conducted at the various fish hatcheries and biological stations. Very little work of this nature has ever been conducted fish living in their normal habitat. Investigations in the field of fish pathology are relatively simple in an aquarium as compared to that found in nature. Such conditions as temperature, reaction, chemical content and oxygen supply can easily be controlled in a trough or pond used in a biological station. This is impossible in rivers, lakes and other inland waters.

Literature concerning this subject reveals only a few cases in which fish were found dying and the causative agent was determined beyond question. In other instances, the investigators have been successful in isolating pathogenic bacteria but have failed to identify them.

Kyle (1) states that fish show remarkable ability in adapting themselves, but like other beings they require time to do this. After a fish has been habituated to a definite habitat it is sensitive to abrupt changes. It seems that fresh water forms are more subject to illness than the marine forms. It is known that they catch cold in the same unaccountable way as do the higher forms of animals. Single

species of fish in a body of water are known to have been swept away completely by epidemics.

Fish are able to adapt themselves to temperature changes providing they are gradual but they are very sensitive to sudden changes. Kyle (1) cites a situation that occurred in 1882 that exemplifies this. A species of marine fish known as the American Tile Fish (Lopholatilus), inhabiting the warm gulf stream off the coast of New England, were almost entirely destroyed by ice water being moved into the area by severe gales from the north.

Kyle (1) also mentions the effects of the outpouring of chemicals into rivers and lakes by sewage disposal. This has had serious effects on fish inhabiting the waters. For example; the salmon has ceased to exist in such European waters as the Thames and Elbe due to these rivers being used for sewage disposal.

An experimental study on the effects of gas wastes upon fishes with special reference to stream pollution was conducted by Dr. Victor E. Shelford (2) of the Illinois State Laboratory of Natural History at Urbana, Illinois. This study revealed some interesting facts. All of the products of destructive distillation of coal thrown wastes into streams and rivers were found to be toxic to fresh water fish. He discovered that fish usually swim away from water that is contaminated with organic matter, such as decomposing

bodies of plants and animals, but they usually swim into gaseous wastes without immediately recognizing them. He also found that the toxicity of gas wastes differed for the different species of fish, and that the toxicity was greater for the smaller and more valuable fish.

Dr. Morris M. Wells (4), of the Illinois State Laboratory of Natural History, made a study of the relation of fresh water fish to acids, salts and alkalies. His findings are as follows; carbonates have no ill effects upon fish providing the water has a mild acid reaction; water that is slightly alkaline lessens the activity of fishes; an N/100 alkalinity of potassium hydroxide (56 parts per million) would prove lethal in a few hours; water that was strictly neutral seemed to have an ill effect upon the common fresh water fish; the best conditions seemed to be a mild acidity; two to six cubic centimeters of carbon dioxide per liter (4-12 parts per million) would furnish the optimum acidity; higher concentrations than this were found to be fatal; fish were observed to live in concentrations of 10 to20 cubic centimeters of carbon dioxide per liter, but this would cause death if they were allowed to remain too long a time in these conditions; an N/10,000 sulphuric acid solution (4.9 parts per million) proved fatal rapidly and when this was diluted down it was found that an N/20,000 sulphuric acid solution (2.4 parts per million) was the optimum acidity for most species of inland fish.

experiment was of little significance.

Some experiments were conducted by Dr. Wiebe (6) at the fish cultural station of Fairport, Iowa, concerning oxygen concentrations and the changes of pH, and their effects on fresh water fish. He found that a high to low concentration of oxygen in water produced unnoticeable effects on fish; the high concentration of oxygen in water being maintained by means of high pressure. There were no indications of injury to the gills of the fish. The low concentration of oxygen, as well, had no ill effects. Fish could survive periods of a month or more at both high and low oxygen concentrations without injurious results. Experiments were carried out on carbon dioxide in a like manner. It was found that fish were capable of tolerating fairly high carbon dioxide concentrations providing there was a correspondingly high concentration of oxygen. Tests were carried out on effects of pH changes in the water on goldfish. They were found to tolerate fairly high acidity as long as the oxygen content was adequate.

Dr. H. S. Davis (7) of the Fairport Iowa Fish Cultural Station, carried out experiments on the effects of vitamin deficiency on fresh water fishes. It was difficult for him to conduct a successful experiment due to the practical impossibility of preventing the fish from getting small quantities of natural food. He used carp, buffalofish, blue-

gill, sunfish and bullhead species in the experiment. Each species of fish were divided into lots and fed diets lacking the various vitamins. Mortality was found to be high in diets completely deficient in vitamins. The most characteristic symptoms of vitamin deficiency occurred in lots deficient in vitamin B. Death usually resulted in this lot when the experiment was protracted. The death rate was high in the lots of fish deficient in vitamin A. It could be assumed, that in a large body of water, fishes were able to secure an adequate source of vitamins.

The habitat of fishes is also an excellent habitat for the many fungi and bacterial parasites. Water that is contaminated with parasites comes in constant contact with the gills and the external surface of the fish. The gills are important invasion sites for bacterial and fungus infections. The alimentary canal, too, is an important portal of entry. Injuries and abrasions on the exterior surface and gills always way for attack by infectious agents. Although the fish displays remarkable powers of resistance to these ever present agents, any diminution of resistance to these changes in environment usually terminates in an infection by one or more of the agents.

It is commonly believed that the usual primary infecting agents are bacteria and the secondary invaders are fungi. This is the usual progression of an infection appearing on

the external surface, gills and fins. However, in most cases, the infection is not conspicuous until the fungus infection appears. Symbiosis of several parasites is evident in most external infections. Sometimes there are two species of bacteria found. More generally it is bacteria and fungus. Generally speaking, the bacteria are the most insidious of the agents since host resistance has been considerably reduced by the time the fungus infection becomes perceptible. Some lesions have been found in which the fungus is the primary agent. The latter is quite often the case when fungus infections are found on external areas previously injured mechanically. Isolation of primary infective agents has been difficult in most investigations undertaken as the secondary invaders and the saprophytes are manifested.

The balance of this survey will deal with some of the most important investigations conducted on external infections, gill infections, infections of the fins and tail, and the internal infections.

There are numerous internal and external animal parasites common to inland fish. They cause a great deal of injury to fish and are very abundant in all inland waters. A discussion of them will be omitted as they are of little importance in this investigation.

A very interesting investigation was conducted by Mr. C.

W. Hahn (8) at a lake in the vicinity of Woods Hole, Massachusetts. This investigation illustrates an infection of fresh water fish by several agents acting in symbiosis. Thickened white and pink areas appeared on the integuments followed by breakdown of structure and excavation of muscle tissue. Fish rapidly succumbed to the disease. Microscopic examinations were made of the diseased tissue using methylene blue and Giemsa's stain. Three organisms of different species were found. The mosy abundant was a short, thick bacillus. A long, slender bacillus was always present, but it was not as numerous as the short bacillus. The third agent found was a sporozoon. Those present were a species of Chloromyxum known as Myxobolus. Hahn believed the primary agent was the long bacillus as this organism was predominant in freshly infected tissue and never appeared in completely atrophied tissue. The short bacillus always appeared in degenerated epidermis and muscle tissue. His report included no attempt, on his part, in isolating and identifying the bacteria.

A new bacterial disease of fresh water fish was found by Dr. H. S. Davis (9), Fish Pathologist of the United States Bureau of Fisheries, while working at the Fairport Iowa Biological Station. This disease was characterized by the appearance of white-yellowish areas on the external surface. These areas rapidly increased until they covered

the entire body with death occurring 24 to 72 hours after the lesions were detected. Most species of game and rough fish were susceptible to infection. Microscopic examination showed the disease to be caused by a species of bacteria, for which Dr. Davis proposed the name Bacillus columnaris, a long, slender, flexible and rod-shaped organism. It was found to be 5 to 12 microns in length and 0.5 microns wide, transparent, motile, and occasionally found in chains. He was unable to culture the organism with the facilities at his disposal. He was also handicapped by lack of proper staining materials and apparatus for determing motility. Failure to isolate the organism rendered it impossible to demonstrate it as the cause of the disease beyond question. There is good evidence of this in the fact that the specific bacteria could always be found in abundance in the diseased tissue. Also, typical lesions could always be produced in healthy fish by scraping off scales in a small area and applying to this the material containing the specific bacteria. No further attempts were made in identifying the the causative organism.

Many types of gill diseases have been found in fresh water fish. While many of these are caused by fungi and animal parasites, a few have been demonstrated to be caused by bacteria. There is one particular gill disease, quite prevalent amongst fresh water fish throughout this country,

that has only recently been recognized as a distinct bacterial infection. There is only one characteristic symptom and that is the appearance of the gills. Generally the fish appears normal until a short time before death. The gills show congestion in the early stages of the disease, then become fused, lighter in color, with tips enlarged. The most notable symptom is the greatly increased secretion of mucus by the gills. A secondary infection of fungus follows in most cases, and is generally present in late stages of the disease. A luxuriant growth of bacteria is always always found when the mucus covering the gills is examined microscopically. They occur in chains of long rod-shaped bacteria and are found most abundant on the outer third of the gill filaments where there is rapid proliferation of the epithelial cells. The gills become necrotic, fungus infection sets in and death follows rapidly. This disease causes heavy losses amongst fresh water fish, especially young fish and fry. There is a rod shaped organism always found in the exudate of gills infected with this disease. Unfortunately, it has never been isolated and identified (10).

There are many external infections in which lesions appear on the fins and tails of fish. It appears that this is a common area for attack by both bacteria and fungi. There is one particular disease of this nature

known as "fin-rot", and it is quite prevalent amongst inland fish, especially those of the trout species. It is characterized by the disintegration and destruction of the fins. The symptoms vary considerably, especially in reference to the particular fin that becomes infected. Sometimes the infection occurs in the tail, and in this instance it is spoken of as being "tail-rot". The pectoral and dorsal fins are the most usual ones to become infected. As a rule, when one fin becomes infected the infection spreads to other fins. The first indication of infection is the appearance of a distinct white line along the outer margin of the fin. The infection gradually moves toward the base and there is a continuous process of disintegration until the entire fin is destroyed. The infective agent is believed to be a rod-shaped organism, observed microscopically in examinations of diseased tissue. The organism has never been identified. The severity of the disease varies greatly and the mortality is quite low (10).

An interesting investigation was conducted by Professor R. C. Osburn (12) of the Department of Zoology of Ohio University, on a disease known as "Black Tumor of Catfish". The disease was found prevalent in a small natural lake near Falmouth, Massachusetts. It is characterized by the appearance of wart-like swellings on the body surface, fins and lips of the catfish. Pressure applied to these

swellings would bring about the extrusion of an inky-black fluid. Microscopic examination of this material with oil-immersion objective, revealed abundant cocci-shaped bacteria having endogenous black pigment. Professor Osburn developed a medium, combining catfish skin tissue and nutrient agar, upon which the organism would grow. Colonies 4 millimeters in diameter were grown in a two week period. Growth occurred at low as well as at high temperatures. These colonies exibited the same black pigment as found in the fluid from the tumors. The organism is one of the few known to secrete an endogenous pigment. It is adequately described by Professor Osburn but was not named. Successful experiments were conducted to determine its pathogenicity. A typical tumor was produced in a healthy catfish when a hypodermic injection was made with fluid containing the organism.

Very few internal infections of fresh water fish have been investigated. By far the most important and most prevalent of these is "Furunculosis". This disease is widespread among both the fresh water and marine forms. It annually takes a heavy toll of fish cultured in the hatcheries and biological stations. The name "Furunculosis" is derived from the most characteristic symptom of the disease, the development of subcutaneous boils on the body of the fish. The first symptom of the disease

is the appearance of red subcutaneous spots followed by a swelling in the area. The swelling is made up of a pus-like fluid containing bacteria, blood and disintegrated tissue. Death has been found to occur in most cases before the disease reaches the stage where boils appear. The causative agent has been found to be a bacterium. It occurs in the blood, causing septicemia, and is now known to be a generalized blood infection. The organism was first described by Emmerick and Weibel in 1894 as the causative agent of a trout epidemic in Germany. They named the organism Bacterium salmonicia. Years later a fish pathologist, by the name of Marsh, isolated the same organism from the blood of an infected fish at Northville, Michigan. He named the organism Bacterium truttae. The two are perhaps identical. Marsh described the organism as a short rod, 2 to 3 microns in length, with rounded ends. The most prominent cultural characteristic described was the formation of a brown pigment that would stain the cultural media brown. Its optimum growth temperature was found to be between 10 and 15 degrees Centigrade. It is believed that the bacteria enter through the digestive tract, gills and small abrasions of the skin. Typical disease has been produced in healthy fish by introducing the organism directly into the blood stream and by infecting the water in which the fish are being cultured. The

process of the disease follows a well defined course from the time the organism is introduced into the blood stream until the red spots appear in the subcutaneous tissue. The visceral organs are especially liable to infection as large numbers of bacteria can always be isolated from these diseased organs. The disease is generally not apparent until the characteristic red swellings appear in the subcutaneous regions of the body. By that time the disease is well advanced and death follows within a few days (10).

Another internal disease of inland fish is "Hemorrhagic- Septicemia." Very little literature is available on this disease. It is believed to be related to a disease of frogs known as "Red-leg".

THEORIES AS TO CAUSE

1. OTHER'S THEORIES.

During the month of January, 1947, several letters were sent to the State Department of Game, Fish and Parks, requesting all available information regarding the Lake Poinsett fish disaster. The nature of this investigation was thoroughly explained in these letters as a reason for securing data. The writer requested permission to study the reports of all inquests made by the department relative to the incident. Request was also made for the theory they supported regarding the cause of death to the fish. Several weeks later a letter was received from the department stating that they believed the disaster was caused by poisonous gases. A letter was then submitted asking what kind of gas it was and what its nature was. This letter has never been answered. Therefore; it is quite evident that the theory supported by the State Department of Game, Fish and Parks is that the fish of Lake Poinsett died from a poisonous gas.

Several members of the Lake Poinsett Region Association were interviewed and the majority of opinions indicate that they believe the fish died of suffocation. One member of this organization believes that the lake became overcrowded with rough fish, thereby reducing the oxygen

content of the water to lethal concentration. The majority of the members interviewed believe the game fish survived the disaster; therefore, they refused to allow the seining of the lake the following winter.

An elderly man, a resident of the locality, stated that a similar tragedy occurred in the lake approximately fifty years ago. There was no positive proof to substantiate his claims.

The writer interviewed a man who is considered one of the best authorities on fishing in South Dakota. This individual has made an extensive study of game fish and their habits. The following is his theory. He believes that the rough fish of Lake Poinsett died of suffocation due to an overcrowded condition induced by a stampede of the carp species of fish. He believes that the heavy cover of snow on the ice, during the winter months preceding the tragedy, excluded the sunlight. Thereby reducing photosynthesis, the normal oxygenating process in the water. The fish became excited, due to lack of oxygen, and stampeded into an area of the lake where they exhausted the oxygen supply completely and died. The game fish, he claims, did not die because they inhabit the deeper water during the winter, and are a less excitable fish than the carp species.

Several residents residing in the lake vicinity support

the above theory with exception that they believe the conditions inducing the suffocation originated in Dry Lake. This, they believe, was followed by a stampede of the fish into Lake Poinsett through a small inlet located at Stone Bridge. Other individuals in this locality believe the fish were at first poisoned in Dry Lake and then stampeded into Lake Poinsett, where they died.

Only a small proportion of the people interviewed expressed belief that the cause was in any way pathological. The majority of people believe the disaster was caused by a reduction of oxygen in the water followed by suffocation.

2. OWN THEORY.

I believe the nature and cause of the death of fish in Lake Poinsett was pathological.

I believe there was an above normal increase in the carbon dioxide content of the water with a consequent lowering of pH. The reaction of the water was moderately acid, varying perhaps between a pH of 6.2 and 6.8. This would have been irritating to the fish but not toxic. This aggravated the fish into a stampede and they congregated in droves in an area of the lake where the water was less irritating. There was a highly infective disease agent present in the water, and with the lowered resistance and close proximity of the fish, this agent was transmitted

rapidly from one individual to another. The decreased resistance of the fish was induced by the acidity of the water and by the stampede. The disease reached epidemic proportions in a very short time and the mortality was high.

I believe the disease was prevalent among the game fish as well as the carp, that a large proportion of both game and rough fish perished, and that it will take years to replenish the stock.

METHODS OF PROCEDURE EMPLOYED

FIELD WORK

1. FIELD WORK DURING FALL OF 1946.

Numerous trips to the lake were made during the months of October and November of 1946, in order to secure samples of dead carp. A small field kit was assembled for this purpose. This contained sterile lancets, forceps, tweezers, rubber gloves, glass containers, and all the necessary items needed in the dissection of the dead fish. All precautions were taken to make the dissections as aseptic as possible. Several friends residing at the lake shore volunteered their services in aiding in the search for specimens. Permission to use a cabin for the dissections was given by one of these residents.

Nine specimens consisting of eight dead carp and one dead bullhead were secured. No samples of dead game fish were found in the search.

No definite pathological lesions were noted in any of the first three dead carp dissected. The spleen, liver, and vascular system of these specimens were placed in one sterile container and the stomach and intestines were placed in another. These were brought to the laboratory immediately for examination.

Methods employed in making the dissection are as follows: The fish were placed over a sterile towel on a table. The

incision was made on the ventral side from the operculum to about one inch behind the anus. The sides were drawn open and tacked with pins to the table. This permitted observation of all internal organs and removal of materials desired for further investigation. The gills and external surface of specimens were carefully scrutinized for pathological lesions before the dissection was performed.

The fourth specimen examined proved very interesting. When the fish was opened, as described above, a thick, viscid, dark brown fluid began oozing out of the abdominal cavity. Uncongealed blood was found in the arteries and veins near the heart. Muscle tissues of the dorsal region of the trunk were found to be colored pink due to uncongealed blood congested in this area. This was considered to be of great significance and care was taken to transfer the fluid material and the organs of the viscera into sterile containers.

The fifth sample dissected was a bullhead. No significant lesions were found and nothing extraordinary was noted. The spleen, liver and other organs of the viscera were removed.

The sixth and seventh samples were both carp. These were found during the last week in October. The first sample dissected was found to be in such an advanced stage of putrefaction that it was discarded. The other dead carp

specimen was found on a lake shore area where the writer had walked only a few hours previously and was therefore believed dead only a short period of time. Interest was increased when it was noted that the same dark viscid fluid and hemorrhagic symptoms appeared in the abdominal cavity of this specimen as had been witnessed previously in one of the others. There was also a darkening of the epidermis on the forward ventral wall noted before the incision was made.

About two weeks later one of the lake residents secured a sample of dead carp, and as the weather was cold, he placed it in a bucket of water and froze it. The sample was thawed out and dissected. A large red swelling was noted in the epidermis surrounding the caudal fin. Nothing of any similarity had been observed in any of the other specimens. The entire fin and surrounding tissue were removed and placed in a sterile container.

The ninth and last sample was secured by the writer during a trip to the lake in the second week in November. This was a dead carp weighing approximately four pounds. Many dead bullheads were also found during this search but they were not dissected. There were no significant characteristics noted in the dissection of this carp sample.

2. FIELD WORK DURING JANUARY, FEBRUARY AND MARCH OF 1947.

Five trips were made to the lake during January, February and March of 1947, in order to secure samples of the lake water for a pH analysis. A box kit containing twelve 16-ounce screw cap bottles was used for carrying water samples to the laboratory for examination. Two samples of water were obtained from Dry Lake and five samples from Lake Poinsett on each of the five trips. One of the Dry Lake samples was always taken near the shore and the other in the center of the lake. Four of the samples from Lake Poinsett were taken at equally distributed points around the lake and the fifth near the center. Holes were cut through the ice with a heavy steel rod. A shovel was carried along in order to remove the snow and slush ice from the area so as not to contaminate the sample. The mouth of the bottle would be covered with the thumb and then held about two feet below the surface of the water and filled. They were capped firmly, labeled and taken to the laboratory for examination. Measurements were made on the depth of the ice and the thickness of the snow covering the ice. Results of this investigation will be given later in the section dealing with laboratory work.

3. FIELD WORK DURING APRIL, 1947.

The 1947 ice cover of Lake Poinsett broke up during the second week of April, approximately a week later than the previous year. Several journeys to the lake were made in order to survey the conditions and make a comparison with those of the previous year.

There were a few dead fish washed into the shores. These were all members of the carp and bullhead species. Several specimens were roughly dissected. Two were found to exhibit the characteristic hemorrhagic symptoms. A live carp, approximately four pounds in weight, was found in the water among the rocks on the lake shore. This specimen appeared to be badly diseased. An advanced stage of fungus infection appeared in the gills. The fish was killed and dissected. No internal lesions or disease symptoms were noted.

Many carp were seen migrating into Lake Poinsett from Dry Lake through the channel at Stone Bridge. A similar process was witnessed in the channel connecting Lake Poinsett with Lake Albert. These fish were reported to be migrating into Lake Poinsett from the Sioux River.

The water in Dry Lake and the northwestern region of Lake Poinsett was extremely turbid, whereas, the water in the southern and eastern regions of Lake Poinsett appeared considerably less turbid.

LABORATORY WORK

1. ISOLATION OF PURE CULTURES.

The samples for examination were taken to the laboratory immediately after they were secured. The materials were placed in sterile dishes and minced. Inoculations of these were made into tubes of nutrient broth and incubated at 37 degrees, Centigrade. The agar pour-plate dilution method was employed. An inoculation was made of the infected material into a tube of melted agar and dilutions were made from this into three successive tubes. These were plated in petri dishes and incubated. Following the period of incubation, the typical isolated colonies were marked. Gram stains were made of these and they were examined microscopically. Those appearing to be pure cultures were inoculated upon agar slants and incubated at 37 degrees Centigrade for 24 hours. These were labeled as to source and a constant record was kept on each.

The alkaline-pyrogallol method was used in removing oxygen for anaerobic growth. After the 24 hour incubation period the nutrient broth tubes were taken from the incubater and streak inoculations were made from these upon a nutrient agar plate. This was inverted upon a partition cup containing pyrogallic acid crystals on one side and a 10% solution of sodium hydroxide on the other. The plate was sealed upon the cup with petrolatum and the chemicals

were mixed. This was incubated for 48 hours at 37 degrees, Centigrade. The isolated colonies were marked and studied microscopically. Those appearing to be pure cultures were isolated and set upon nutrient agar plates and maintained under the same anaerobic conditions.

All of the materials brought in for examinations from the lake were handled in the same manner as described above. The isolated pure cultures were transferred to fresh media every 48 to 72 hours to prevent dissociation. When time permitted, complete cultural and physiological examinations were made before the organism had a chance to lose vigor and become attenuated.

2. LABORATORY STUDIES OF THE MORPHOLOGICAL, CULTURAL, AND PHYSIOLOGICAL CHARACTERISTICS OF THE ISOLATED PURE CULTURES.

Gram stains were made of each culture and the morphological characteristics were determined by microscopic examination.

Cultural characteristics were determined by examination of growth on agar plates, agar slants and nutrient broth. They were then grown in a variety of differential media to determine their physiological characteristics.

The results of these examinations are compiled in the following tables.

TESTS		PURE CULTURE NO. 1	PURE CULTURE NO. 2
Source		Isolated from dead carp No. 4	Isolated from dead carp No. 4
MORPHOLOGICAL	Shape	rod shaped	Short rods, almost coccoid.
	Size	0.5 by 2.5 microns	0.5 by 1.5 microns
	Motility	Motile	Motile
	Cell arrangement	Singly and in chains	Singly and in pairs
	Spores	Spores - central	No spores formed
	Gram stain	Gram positive	Gram negative
	Oxygen requirements	Aerobic	Aerobic
CULTURAL	Agar colonies	Large, spreading, convex, grayish in color, amoeboid margin	White, 2 to 3 mm. in diameter, moist and glistening, entire, undulate and flat.
	Agar slant	Beaded at first, growth thin, whitish in color, spreading.	White, moist, glistening, spreading, fecal odor produced.
	Broth	Heavy turbid growth, pellicle formed, no odor.	Turbid, some sediment, no pellicle, fecal odor.
PHYSIOLOGICAL	Litmus milk	Alkaline, not coagulated, no gas, no wheying off, slowly peptonized.	Acid, reduction, coagulation with development of gas furrows, no proteolysis.
	Starch agar	Hydrolysis	No hydrolysis
	Gelatin	Abundant stratiform liquefaction	No liquefaction
Sugar broths with Durham fermentation tubes	Dextrose	Acid, no gas	Acid and gas
	Sucrose	Slightly acid, no gas	Acid and gas
	Lactose	No change	Acid and gas
	Nitrate agar	Nitrates are not reduced.	Nitrates are reduced to nitrites.
	Tryptone broth	Indol is not formed	Indol is formed
	Fresh bloodagar	No hemolysis	No hemolysis

TESTS		PURE CULTURE NO. 3	PURE CULTURE NO. 4
Source		Isolated from dead carp No. 3	Isolated from dead carp No. 2
MORPHOLOGICAL	Shape	Short rods	Short rods
	Size	0.5 by 1.5 microns	0.5 by 2.0 microns
	Motility	Non-motile	Motile
	Spores	No spores formed	No spores formed
	Cell arrangement	Singly and in pairs	Singles and pairs
	Gram Stain	Gram negative	Gram negative
	Oxygen requirements	Aerobic	Aerobic
CULTURAL	Agar colonies	Thick, white, moist, raised, convex, circular, 5 mm. diameter, margin is entire.	Grayish in color, spreading, large, irregular colonies.
	Agar slant	Thick, white, moist, spreading, glistening, filiform growth heavy.	Thin growth, bluish, spreading.
	Broth	Pellicle formed, turbid with sediment.	Slight sediment, pellicle, turbid.
PHYSIOLOGICAL	Litmus milk	Acid, coagulation and wheying off, gas furrows, no reduction, no peptonization,	Rapid peptonization, acid at first then alkaline, no coagulation.
	Starch agar	No hydrolysis	No hydrolysis
	Gelatin	No liquefaction	Rapid stratiform liquefaction.
Sugar broths with Durham fermentation tubes	Dextrose	Acid and gas	Acid and gas
	Sucrose	Acid and gas	Acid and gas
	Lactose	Acid and gas	No change
	Nitrate agar	Nitrates reduced to nitrites.	Nitrates reduced to nitrites.
	Tryptone broth	Indol is not formed	Indol is not formed
	Fresh blood agar	No hemolysis	No hemolysis
	Lead acetate agar		Hydrogen sulphide is formed.

TESTS		PURE CULTURE NO. 5	PURE CULTURE NO. 6
Source		Isolated from dead carp No. 4	Isolated from dead carp No. 1
MORPHOLOGICAL	Shape	Short rods	rods
	Size	0.5 by 1.5 microns	1.5 by 4 microns
	Motility	Motile	Motile
	Spores	No spores formed	Spores - central
	Cell arrangement	Singles, pairs, and short chains.	Singles, pairs, short and long chain
	Gram Stain	Gram negative	Gram positive
	Oxygen requirements	Aerobic - Facultative	Aerobic
CULTURAL	Agar colonies	Beaded at first, round whitish, raised, becoming brown, entire.	White and grayish, spreading rapidly, filamentous, rhizoid
	Agar slants	Beaded at first then whitish, glassy and spreading, brownish color with age.	White, glistening, rhizoid, rapidly spreading over entir surface.
	Broth	Turbid, sediment, heavy pellicle ring.	Turbid, slight pellicle, light sediment.
PHYSIOLOGICAL	Litmus milk	Acid, coagulation, slight reduction, peptonization.	Alkaline, no coagulation, no reduction proteolysis.
	Starch agar	Hydrolysis	No hydrolysis
	Gelatin	Rapid liquefaction, napiform.	Liquefaction, tubula or saccate in form.
Sugar broths with Durham fermentation tubes	Dextrose	Acid and gas	Acid, no gas
	Sucrose	Acid and gas	Acid, no gas
	Lactose	No change	No change
	Nitrate agar	Nitrates are reduced to nitrites.	Nitrates are not reduced.
	Tryptone broth	Indol is formed	Indol is not formed
	Fresh blood agar	Beta hemolysis, 3 to 4 centimeters in 24 hours.	No hemolysis
	Lead acetate agar	Hydrogen sulphide is not formed.	

TESTS		PURE CULTURE NO. 7	PURE CULTURE NO. 8
Source		Isolated from dead carp No. 7	Isolated from dead carp No. 7
MORPHOLOGICAL	Shape	Short rods	Short rods
	Size	0.5 by 1.5 microns	0.5 by 1.5 microns
	Motility	Motile	Motile
	Spores	Spores are not formed.	Spores are not forme
	Cell arrangement	Singles, pairs and short chains.	Singles and pairs
	Gram Stain	Gram negative	Gram negative
	Oxygen requirements	Aerobic	Aerobic
CULTURAL	Agar colonies	Raised, entire, white, brown with age.	Flat, white, entire, moist, glistening.
	Agar slant	Thin growth, white, spreading, brown with age.	White, moist and spreading.
	Broth	Turbid with a ring pellicle, slight sediment.	No pellicle formed, turbid with slight sediment.
PHYSIOLOGICAL	Litmus milk	Reduction, acid and coagulation, rapid peptonization.	No peptonization, acid with coagulation gas bubbles in whey.
	Starch agar	Hydrolysis	No Hydrolysis
	Gelatin	Rapid napiform liquefaction.	No liquefaction
Sugar broths with Durham fermentation tubes	Dextrose	Acid and gas	Acid and gas
	Sucrose	Acid and gas	Acid and gas
	Lactose	No change	Acid and gas
	Nitrate agar	Nitrates are reduced to nitrites.	Nitrates are reduced to nitrites.
	Tryptone broth	Indol is formed	Indol is formed
	Fresh blood agar	Hemolysis, an area of 2 centimeters	No hemolysis

TESTS		PURE CULTURE NO. 9	PURE CULTURE NO. 10
Source		Isolated from dead carp No. 7	Isolated from dead carp No. 7
MORPHOLOGICAL	Shape	Short rods	Spheres-cocci
	Size	0.5 by 1.5 microns	0.7 microns diameter
	Cell arrangement	Singles, pairs and short chains.	Singly and in pairs
	Motility	Motile	Non-motile
	Spores	Spores are not formed	No spores formed
	Gram Stain	Gram negative	Gram positive
	Oxygen requirements	Aerobic-facultative	Aerobic
CULTURAL	Agar colonies	White, spreading, flat, translucent, circular, entire, somewhat undulate.	Small, granular, dull flat, amoeboid, orange in color.
	Agar slant	White, spreading, fecal odor.	Orange, undulate, flat and spreading.
	Broth	Turbid, sediment, no pellicle, fecal odor.	Turbid, sediment and a light pellicle.
PHYSIOLOGICAL	Litmus milk	Alkaline, no coagulation, no reduction, no peptonization.	Acid, coagulation, and reduction.
	Starch agar	Hydrolysis	No hydrolysis
	Gelatin	No liquefaction	Stratiform liquefaction
Sugar broths with Durham fermentation tubes	Dextrose	Acid and gas	Acid and gas
	Sucrose	Acid and gas	Acid and gas
	Lactose	Acid and gas	Acid but no gas
	Nitrate agar	Nitrates reduced to nitrites.	Nitrates are not reduced.
	Tryptone broth	Indol is not formed	Indol is not formed
	Fresh Blood agar	No hemolysis	No hemolysis

TESTS		PURE CULTURE NO. 11	PURE CULTURE NO. 12
Source		Isolated from dead carp No. 8	Isolated from dead carp No. 9
MORPHOLOGICAL	Shape	Spheres, cocci shape	rod shaped
	Size	1 to 2 microns in diameter.	1 by 4 microns
	Motility	Non-motile	Motile
	Spores	No spores formed	Spores - central
	Cell arrangement	Regular packets of 4 and 8.	Singly, pairs and short chains.
	Gram Stain	Gram positive	Gram positive
	Oxygen requirements	Anaerobic	Aerobic
CULTURAL	Agar colonies	Beaded at first, slightly raised and irregular, yellow, granular, wrinkled.	Round, entire, white colonies, thick, opaque.
	Agar slants	Very little growth, thin yellow film in time.	White, moist, spreading, filiform growth.
	Broth	Slight turbidity, no pellicle, slight sediment.	No pellicle, turbid, slight sediment.
PHYSIOLOGICAL	Litmus milk	Becomes slightly alkaline, no reduction no proteolysis.	Reduction, alkaline, some peptonization.
	Starch agar	No hydrolysis	No hydrolysis
	Gelatin	No liquefaction	Craterform liquefacti
Sugar broths in Durham fermentation tubes	Dextrose	No change	No change
	Sucrose	No change	No change
	Lactose	No change	No change
	Nitrate broth	Nitrates are not reduced.	Nitrates are not reduced.
	Tryptone broth	Indol is not formed.	Indol is not formed.
	Fresh blood agar	Very slight hemolysis in 48 hours	No hemolysis

3. IDENTIFICATION AND DISCUSSION OF PURE CULTURES.

Pure Culture No. 1

This organism is definitely identified from its characteristics as being *Bacillus subtilis*. It is non-pathogenic and is therefore of no significance in this investigation. The normal habitat of this organism is soil and water.

Pure Culture No. 2

The above characteristics of No. 2 organism identify it as a coliform. It is *Escherichia coli* and is differentiated from other coliforms by its production of indol in tryptone broth, and the cultural characteristics of its agar colonies. Its normal habitat is the intestines of man and animals. It has no significance in this discussion.

Pure Culture No. 3

This organism is a coliform and is identified as *Aerobacter aerogenes*. It is differentiated from *E. coli* by its inability to form indol in tryptone broth, and by its raised colonies on agar plates. It is insignificant in this investigation as it is common in soil water and dust. It is non-pathogenic. It was considered unnecessary in this investigation to use either methyl red or Voges-Proskauer tests in differentiating the coliforms.

Pure Culture No. 4

This organism is identified as a member of the Proteus group by its characteristic proteolytic action. It was necessary to grow it on lead acetate agar to definitely identify it as Proteus vulgaris. This organism is commonly found in putrefying bodies of animals. It is a saprophyte, generally non-pathogenic, and is of no significance in this investigation.

Pure Culture No. 5

This organism is a member of the Proteus group. It was at first believed to be Proteus vulgaris or a strain of this bacteria. The differentiation of reaction in litmus milk, formation of indol, hemolysis of blood agar, and the distinctively different agar colony characteristics were sufficient to convince the investigator that this organism was not Proteus vulgaris. It was definitely identified as being Proteus hydrophilus. It was isolated from the dark viscid fluid found in the abdominal cavity of dead carp No. 4. It was believed to be pathogenic by its hemolysis of blood agar.

Pure Culture No. 6

The organism of this culture was readily identified by microscopic examination and its characteristic growth on agar plates as being Bacillus mycoides. The other tests

furnished conclusive proof in the identification. It is a common inhabitant of the soil and is of no significance in this investigation.

Pure Culture No. 7

The characteristics of this organism are identical to those of Proteus hydrophilus, the same as culture No. 5. This culture was isolated from the hemorrhagic material found in the abdominal cavity of dead carp No. 7. It is very significant.

Pure Culture No. 8

The cultural and physiological characteristics of this organism readily identify it as E. coli. It is of no significance.

Pure Culture No. 9

This organism is classified as a coliform by its reaction in litmus milk, lactose broth and gelatin. It is differentiated from E. coli by its inability to produce indol in tryptone broth and by its alkalinizing of litmus milk. It appears to be Escherichia alkalescens, a normal inhabitant of the intestines of man and animals. It has no significance.

Pure Culture No. 10

Characteristics of this organism indicate that it is

either Micrococcus perflavus or Micrococcus flavus liquefaciens. Its normal habitat of the air and soil is indicative that it has no significance in this investigation.

Pure Culture No. 11

This was the only true anaerobic organism isolated from the dead fish. The cubical packets of its cell arrangement distinguishes it as a member of the Sarcina group. Bergey lists several Sarcina species that are anaerobic. They are Sarcina methanica, Sarcina maxima, and Sarcina beijerinckii. The organism of culture No. 11 resembles Sarcina beijerinckii in characteristics morphologically and cultural. But there are several physiological characteristics such as action in sugar broths and litmus milk that definitely differentiate the two organisms. It apparently does not correspond to any organism listed in Bergey's Manual of Determinative Bacteriology. This unknown Sarcina organism of culture No. 11 is a pathogen and is therefore significant.

Pure Culture No. 12

The characteristics of this organism agree with those of Bacillus fusiformis, a normal inhabitant of soil and water. It has no significance.

4. TESTS FOR PATHOGENICITY.

A number of common goldfish were secured for the pathogenicity tests. These fish were about 6 to 8 inches in length and were of the hardiest species. The fish were ascertained to be disease free before they were used in the tests.

Three glass jars were secured. Tap water and aquarium plants were added to these approximately 24 hours before the fish were placed in them in order that the water be at room temperature. The jars were labeled Control, No. 1 and No. 2. After the fish were added, they were allowed to stand for several days to make certain the fish showed no ill effects before the tests were started.

The organisms of pure culture No. 5 (Proteus hydrophilus) and No. 11 (the unknown Sarcina) were grown upon blood agar plates for 48 hours and then inoculated into tubes containing 5 milliliters of nutrient broth. These tubes were incubated at 37 degrees Centigrade for a period of 24 hours. One milliter of the broth containing Proteus hydrophilus was inoculated into jar No. 1 and one milliter of the broth containing the unknown Sarcina was inoculated into jar No. 2. The three jars were placed on a table in the laboratory where they would be in no danger of contamination. The fish were fed every 24 hours, care being taken to prevent overfeeding.

The water of test jar No. 1 (inoculated with Proteus hydrophilus) became turbid in 24 hours. The fish appeared distressed, remaining near the surface. The water developed a noticeable foul odor by the third day and the fish showed signs of general weakness. A gradual general weakening was evident during the following days. Food was completely neglected and there was no response to stimulation. Death occurred on the seventh day.

The water in test jar No. 2 was mildly turbid by the third day. The fish exibited no ill effects during this period and there was no foul odor evident. This fish appeared normal in all respects and continued to take food until the ninth day. Evidence of a weakened condition and lessened activity was noted on the tenth day. Death occurred on the 12th day.

The fish in the control jar showed absolutely no ill effects during this period of time. It remained living in the same jar for several months following the experiment.

The dead goldfish was taken from test jar No. 1 and immediately dissected as aseptic as possible. The characteristic dark viscid hemorrhagic fluid was found in the abdominal cavity. Uncongealed blood was found in the blood vessels near the heart. The muscle tissues of the trunk were a reddish-pink color. Inoculations of hemorrhagic fluid were made into nutrient broth and a tube of melted agar. Dilu-

tions were made with two other tubes and these were poured into sterile petri dishes. These were incubated for 24 hours at 37 degrees, Centigrade. Typical colonies were picked from the agar plates and inoculated on agar slants. The results of the tests conducted on the isolated pure cultures are shown on a table below.

No evidence of internal pathological lesions were noted when the dead goldfish from test jar No. 2 was dissected. Inoculations were made from the liver and spleen into broth tubes. A small red swelling was noted below the epidermis near the pelvic fin. The epidermis was carefully removed and material from the area was inoculated into tubes of nutrient broth and tubes of melted agar for dilution pour-plates. These were incubated at 37 degrees Centigrade for 24 hours. Inoculations were made from the broth cultures upon agar plates and these were sealed upon a partitioned cup containing pyrogallic acid crystals and 10% sodium hydroxide solution to furnish anaerobic growth conditions. The characteristic colonies with yellow pigment were noted after a 48 hour incubation period.

Gram stains were made of the pure cultures isolated from the dead goldfish of No. 1 test jar. Three of these cultures were found to be short gram negative rods and these were subjected to the identification tests. The following table show the results and the identification follows.

	TESTS	AGAR SLANT CULTURE NO.1	AGAR SLANT CULTURE NO. 2	AGAR SLANT CULTURE NO. 3
MORPHOLOGICAL	Shape	Short rods	Short rods	Short rods
	Size	0.5-1.5 microns	0.5-1.5 microns	0.5-1.5 microns
	Motility	Motile	Motile	Motile
	Cell arrangement	Singles, pairs, short chains.	Singles, pairs, short chains.	Singles and pairs.
	Gram Stain	Gram negative	Gram negative	Gram negative
	Oxygen	Aerobic	Aerobic	Aerobic
CULTURAL	Agar colonies	Raised, entire, white becoming brown with age.	Raised, entire, white becoming brown with age	Flat, glistenin white, fecal od
	Agar slant	White, spreading, translucent, becomes brown.	White, spreading becoming brown.	White, spreadin fecal odor.
	Broth	Turbid, pellicle sediment.	Turbid, pellicle sediment.	Turbid, fecal odor, sediment.
PHYSIOLOGICAL	Litmus milk	Peptonization, reduction, acid and coagulation.	Peptonization, reduction, acid and coagulation.	Acid, gas furrow coagulation, n peptonization.
	Starch agar	Hydrolysis	Hydrolysis	No hydrolysis
	Gelatin	Napiform liquefaction	Napiform liquefaction	No liquefaction
	Dextrose	Acid and gas	Acid and gas	Acid and gas
	Sucrose	Acid and gas	Acid and gas	Acid and gas
	Lactose	No change	No change	Acid and gas
	Nitrate agar	Nitrates are reduced to nitrites.	Nitrates are reduced to nitrites.	Nitrates are reduced to nitrites.
	Fresh blood agar	2 centimeters hemolysis	2 to 3 centimeters hemolysis	No hemolysis

The agar slant cultures No. 1 and 2, isolated from dead golffish of jar No. 1, are Proteus hydrophilus beyond doubt. The No. 3 slant culture is E. coli. Therefore, by isolating this organism from the diseased tissue, it is certified that it is the causative agent of the septicemic disease of fish.

Robert Koch formulated his classical postulates on the requirements necessary to incriminate a specific bacteria as the causative agent of a specific disease. His four postulates are as follows: (13)

(1) The organism should always be associated with the disease.

(2) The organism must be isolated in pure culture.

(3) The organism should, in pure culture, reproduce the disease in a healthy susceptible animal.

(4) It must be isolated in pure culture from the lesions of the inoculated animal.

The requirements, as outlined in Koch's postulates, have been met in this disease. These facts prove beyond doubt that Proteus hydrophilus is the causative agent of the hemorrhagic septicemia disease of fish.

Only strictly anaerobic pure cultures isolated from the dead goldfish of jar No. 2 were examined. The typical isolated yellow pigmented colonies on the anaerobic plate were marked and two of them were subjected to the identification tests. The results of these tests are presented in the following table.

TESTS		COLONY NO. 1	COLONY NO. 2
MORPHOLOGICAL	Shape	Spheres - cocci	Spheres - cocci
	Size	1 to 2 microns in diameter.	1 to 2 microns in diameter.
	Motility	Non-motile	Non-motile
	Spores	No spores formed	No spores formed
	Cell arrangement	Regular packets of 4 and 8.	Regular packets of 4 and 8.
	Gram Stain	Gram positive	Gram positive
	Oxygen requirements	Anaerobic	Anaerobic
Cultural	Agar colonies	Raised, irregular, yellow pigment, becomes wrinkled and granular.	Raised, irregular, yellow pigment, becomes wrinkled and granular.
	Agar slant	Very thin yellowish growth.	Very thin yellowish growth.
	Broth	Very slight turbidity with sediment.	Very slight turbidity with sediment.
Physiological	Litmus milk	Alkaline no proteolysis	Alkaline no proteolysis
	Starch agar	No hydrolysis	No hydrolysis
	Gelatin	No liquefaction	No liquefaction
	Dextrose	No change	No change
	Sucrose	No change	No change
	Lactose	No change	No change
	Nitrate agar	Nitrates are not reduced.	Nitrates are not reduced.
	Tryptone broth	Indol is not formed	Indol is not formed

This organism is the same as the unknown Sarcina originally inoculated into No. 2 jar. The requirements of Koch's postulates have also been met in this case. This organism is the causative agent of the red subcutaneous swelling occurring near the fins of fish. The organism was also isolated from the spleen of the goldfish so it is presumed to be a blood disease. It does not correspond to the bacteria that causes Furunculosis. Marsh recently isolated the causative organism for the disease Furunculosis at Northville, Michigan, and the bacteria that he found was rod-shaped. It is possible this organism has never been isolated previously.

This disease was found in only one specimen taken from Lake Poinsett. It is not believed to be of any great significance even though it was fortunate to have accidentally discovered it in this investigation. So far as the cause of the disaster is concerned, the hemorrhagic septicemia disease caused by Proteus hydrophilus is believed to be by far the most significant of the two.

5. pH ANALYSIS OF THE LAKE WATER.

The five groups of water samples secured from the lake were brought to the laboratory for pH determinations. The first group of the samples, taken from the lake on January 17th, were tested calorimetrically. A new Beckman pH meter, belonging to the South Dakota State College Mechanical Engineering Department, was used in the determinations of the last four groups. This apparatus simplified the procedure considerably and it was believed to be a more accurate method. The following table shows the dates the samples of water were taken and the region of the lake where they were secured.

pH Of The Water Samples

DATE THE SAMPLE WAS SECURED	100 feet from shore of Dry Lake	Center of Dry Lake	North west side of Lake Poinsett	South west side of Lake Poinsett	South east side of Lake Poinsett	North East side of Lake Poinsett	Center of Lake Poinsett
January 17th	6.7	6.8	6.8	6.9	6,8	6.9	6.9
February 2nd	6.7	6.8	6.8	6.9	6.9	6.9	6.9
February 16th	6.6	6.8	6.8	6.8	6.9	6.7	6.8
March 2nd	6.5	6.7	6.75	6.9	6.8	6.8	6.8
March 16th	6.5	6.6	6.7	6.8	6.8	6.8	6.8

The above data shows that the water in Lake Poinsett has a slight acid reaction during the winter when it is covered with ice. Neglecting any possible errors in handling and inaccuracy of the apparatus, the above figures indicate that the acidity of the water increased slightly during this period.

Rough measurements were made of the depth of the snow and the thickness of the ice during this period. The thickness of the ice varied between 22 and 34 inches. The depth of snow on the ice varied all the way from 0 to about 12 inches. There was no relationship between the depth of snow and ice and the acidity of the water beneath it.

The data above shows that the acidity of the water in Dry Lake and the Northwestern Lake Poinsett region increased slightly more than that of other areas. This was perhaps due to more decomposition of organic matter in this area of the lake. This is quite significant as it was perhaps in this area that the disturbance originated the preceding year.

Whether or not the pH values of the lake water during the winter of 1945-1946 were the same as these is only a conjecture. It is believed that the water may have been slightly more acid perhaps due to a greater amount of organic decomposition.

6. BACTERIOLOGICAL EXAMINATION OF THE LAKE WATER.

Several of the samples of lake water brought in to the laboratory for pH determinations were also examined bacteriologically. A search was made of these samples for the presence of Proteus hydrophilus, the pathogenic bacteria found in this investigation.

Dilutions of the water samples were made using three 99 milliter sterile water blanks. Inoculations were made into melted agar tubes and these were poured into sterile petri dishes making dilutions of 1 to 100, 1 to 1,000 and 1 to 10,000 respectively. The plates were incubated at 37 degrees Centigrade for 48 hours.

There were no pathogenic organisms, including Proteus hydrophilus, found in any of the water examples examined.

7. DETERMINATION OF THE OPTIMUM GROWTH TEMPERATURE OF PROTEUS HYDROPHILUS.

Facilities for growing bacteria cultures at various ranges of temperature were secured at the South Dakota State College Dairy Department, Pharmacy Department, and Bacteriology Department.

Eight agar slants were inoculated with Proteus hydrophilus, divided up into four sets, and handled as follows. One set was placed in a refrigerator with a constant tem-

perature of 5 degrees, Centigrade. The second set was placed in a refrigerator with a temperature of 15 to 20 degrees, Centigrade. The third set was placed in the incubator at 37 degrees, Centigrade. The fourth set was placed in a constant temperature water bath at 45 degrees, Centigrade.

All of the agar slants were examined after a 48 hour period. Those that had been placed in the 15 to 20 degree Centigrade range showed the greatest amount of growth. Those placed in the 37 degree Centigrade range were second. There was no growth on the agar slants placed in the 45 degree Centigrade temperature water bath. A small amount of growth was found on the agar slants placed in the 5 degree Centigrade range.

The optimum growth temperature for Proteus hydrophilus appears to be between 15 and 20 degrees, Centigrade. The fact that the organism will grow at temperatures as low as 5 degrees Centigrade is very significant.

CONCLUSIONS

1. There has been no way of determining what percent of the fish of Lake Poinsett died during the disaster. Perhaps a rough estimate could have been made if the lake had been seined during the winter of 1946-1947.

2. Conclusive information concerning the disposition of the game fish is lacking. An answer to this question could also have been secured if the lake had been seined. Concerning the apparent absenteeism of game fish, the following evidence supports belief that they died in the disaster.

(1) There have been no game fish angled, so far as the investigator has been able to determine. All rumors of this nature have been investigated and found not to be authentic.

(2) The carcass of a dead pike was drawn from the bottom of the lake by a fisherman while casting at the lake during the summer of 1946. This supports the belief that the dead game fish sank to the lake bottom.

(3) The air-sac of the game fish deteriorates rapidly after death, thus causing the fish to sink to the bottom of the lake. This process does not take place as rapidly in the carp. The carp species will float on the surface for a long time before putrefaction breaks down the membranes of this buoyant organ.

3. The writer believes that there was a sufficient supply of oxygen in the water of Lake Poinsett, prior to the disaster, to adequately support the life of the fish. Several cases are known where large numbers of rough fish have survived a winter in a small pool without ill effects. Considering the tremendous quantity of water contained in Lake Poinsett, it seems reasonable to believe that there should be sufficient oxygen to supply the requirements of the fishes that normally have a much lower metabolism during the winter months.

The exclusion of sunlight by snow and ice is not believed to be of any significance. It was noted in this investigation that there appeared to be no relationship between the depth of the snow and ice and the acidity of the water. Only a very little photosynthesis is believed to take place in lake water during the winter. If the fish depended upon this process for their oxygen supply during the winter then the fish in all of the inland lakes would die.

4. Dr. Shelford (2) found that the best reaction of water for the fishes was a mild acidity; therefore, the water of Lake Poinsett, with a pH of 6.7 to 6.9 appears to be ideal.

The pH analysis of the lake water indicates a very slight increase of acidity during the period the lake was covered with ice. This is believed to be normal for all of the lakes

in the region. A pH of 6.5, as found in Dry Lake, would either be mildly irritating or have no effect at all on the fish. A considerable quantity of carbon dioxide is necessary to lower the pH a tenth. Considering the quantity necessary to lower the pH to toxic levels, it seems that toxic acidity could not have caused the disaster. However, sufficient carbon dioxide gas could have been produced in the region of Dry Lake to have become irritating to the fish. It is doubtful if all the water of Lake Poinsett would have reached even an irritating level.

5. Decomposition of organic matter took place, to some extent, in both Dry Lake and Lake Poinsett during the period preceding the disaster. Considerable decomposition took place during the year following the disaster, yet the pH of the water did not go below 6.5. There is also reason to believe that as much decomposition of plant matter took place in these lakes during the years preceding the disaster. Considerable plant decomposition took place in Lake Albert, the lake adjoining Lake Poinsett, yet the fish in that lake lived.

The most usual gaseous products of decomposition of this kind are carbon dioxide, methane, hydrogen, ammonia and hydrogen sulphide. Methane and hydrogen are non-toxic to fish. Hydrogen sulphide is very toxic but as a rule is

found only in waters used for sewage disposal. Lake Poinsett has never been used for this purpose. Ammonia gas is toxic to fish providing it is present in high concentration. Tremendous quantities of this gas would have had to be produced to effect the entire lake. One could believe, without too much restraint, that poisonous gases produced by organic decomposition could kill fish in a small body of water. But this seems unbelievable in such a tremendous quantity of water as is contained in Lake Poinsett, the largest lake within South Dakota.

6. This investigation has revealed the presence of a hemorrhagic septicemia disease in the fish of Lake Poinsett. The specific causative organism of this disease has been demonstrated to be Proteus hydrophilus. This disease was found in several of the specimens of dead carp taken from the lake. It has been verified that it is a communicable disease, capable of being transmitted in water from one fish to another. It is very infective, insidious, and capable of causing death of fish in a short period of time. The capability of the organism to grow at low temperatures has been demonstrated.

Here, then, is an organism pathogenic to fish, and producing symptoms so like those seen previously in the Lake Poinsett disaster that it may well have been the cause, or one of the causes of the disaster.

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I wish to express my appreciation and thanks to Prof. Arthur Grismer of the South Dakota State College Bacteriology Department for technical advice and the helpful suggestions he has given me in this research project.

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